

ATTORNEY DOCKET NO. 07038.0002
PATENTIN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
Leskovar, P.) Group Art Unit: 1642
Serial No. 08/808,334) Examiner: Ungar, S.
Filed: February 28, 1997)
For: DRUGS FOR TREATING CANCER,)
AIDS, AND VIRAL DISEASES)

REVOCATION AND SUBSTITUTE POWER OF ATTORNEY

Assistant Commissioner for Patents
Washington, D.C. 20231

NEEDLE & ROSENBERG, P.C.
Suite 1200, The Candler Building
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Atlanta, Georgia 30303-1811

December 1, 1998

Sir:

Peter Leskovar hereby revokes all powers of attorney heretofore given by him and
appoints as his attorneys:

William H. Needle (Reg. No. 26,209); Sumner C. Rosenberg (Reg. No. 28,753); David G. Perryman (Reg. No. 33,438); Mitchell A. Katz (Reg. No. 33,919); Gregory J. Kirsch (Reg. No. 35,572); Gwendolyn D. Spratt (Reg. No. 36,016); Negandra Setty (Reg. No. 38,300); D. Andrew Floam (Reg. No. 34,597); Clark G Sullivan (Reg. No. 36,942); Mary L. Miller (Reg. No. 39,303); Bryan W. Bockhop (Reg. No. 39,613); Bradley K. Groff (Reg. No. 39,695); Kean J. DeCarlo (Reg. No. 39,954); Elket V. Swope (Reg. No. 40,195); Allan G. Altera (Reg. No. 40,274); LaVonda DeWitt (40,396); Mark A. Westhafer (Reg. No. 42,220); Janice A. Kimpel (Reg. No. 42,734); Mark A. Murphy (42,915); Matt Josephic (P-43,699); Tina McKeon (P-43,791); and Larry A. Villaneuva (P-43,968).

ATTORNEY DOCKET NO. 07038.0002
SERIAL NO. 08/808,334

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with full power of substitution, association and revocation, to prosecute said application and to
transact all business in the Patent and Trademark Office connected therewith.

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March 3, 1999

Date

Dr. Peter Leskovar

Peter Leskovar
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Citizen of Germany

- 2 -

Dr. Peter Leskovar

(Rosenheim
March 3, 1999)

DECLARATION, POWER OF ATTORNEY AND
APPOINTMENT OF DOMESTIC REPRESENTATIVE

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

DRUGS FOR TREATING CANCER, AIDS AND VIRAL DISEASES
the specification of which

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to be material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, 1.56 and 1.63(d).

I hereby claim foreign priority benefits under Title 35, United States Code, 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority <u>Claimed</u>
PCT/EP89/ 00403 (Number)	EPO (Country)	14/4/89 (Day/Month/Year Filed)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
P38 12 605.2 (Number)	Germany (Country)	15/4/88 (Day/Month/Year Filed)	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

I hereby claim the benefit under Title 35, United States Code, 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in

the manner provided by the first paragraph of Title 35, United States Code, 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, 1.56 and 1.63(d) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.) (Filing Date) (Status) (Patented, Pending, Abandoned)

(Application Serial No.) (Filing Date) (Status) (Patented, Pending, Abandoned)

POWER OF ATTORNEY

I hereby appoint THEODORE R. PAULDING, Registration No. 19,294 - DONALD K. HUBER, Registration No. 18,686 - JOHN C. HILTON, Registration No. 22,965 - FREDERICK J. HAESCHE, Registration No. 24,529 - JOHN C. LINDERMAN, Registration No. 24,420 - JACK M. PASQUALE, Registration No. 31,052, J. KEVIN GROGAN, Registration No. 31,961, JOSEPH S. KENTOFFIO, Registration 33,189, JOSEPH A. FISCHETTI, Registration No. 32,656, F. TYLER MORRISON III, Registration No. 36,220 and MARK D. GIARRATANA, Registration No. 32,615; all of the firm of McCORMICK, PAULDING & HUBER, CityPlace II, 185 Asylum Street, Hartford, Connecticut 06103-4102, Telephone No. (203) 549-5290, as my attorneys to prosecute this application, to make alterations and amendments therein, to receive the patent and all correspondence relating to this application, and to transact all business in the U. S. Patent and Trademark Office connected therewith, and the said attorneys are hereby given full power of substitution and revocation.

APPOINTMENT OF DOMESTIC REPRESENTATIVE

The above-identified attorneys, also known as McCORMICK, PAULDING & HUBER, whose postal address is CityPlace II, 185 Asylum Street, Hartford, Connecticut 06103-4102, United States of America, are hereby designated applicant's representative upon whom notices or process in proceedings affecting the patent may be served. Said firm shall take instructions from my foreign patent agents in all matters affecting this application and the patent.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may

jeopardize the validity of the application or any patent issued thereon.

Peter Leskovar

Full name of sole or first inventor

Peter Leskovar

Inventor's Signature

26th April 1994 German

Date Citizenship

83026 Rosenheim, Tizianstr. 3c

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Inventor's Signature

Date Citizenship

Residence Address

Post Office Address

ATTORNEY DOCKET NO. 07038.4003U2
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
Leskovar, P.) Group Art Unit: Unassigned
Serial No. Unassigned) Examiner: Unassigned
Filed: Unassigned)
Continuation of Serial No. 09/302,642)
Filed: May 3, 1999)
For: "DRUGS AND METHODS FOR)
TREATING CANCER")

DECLARATION OF PETER LESKOVAR UNDER 37 C.F.R. § 1.132

I, Peter Leskovar, a citizen of Germany, residing at D-83026 Rosenheim, Tizianstr.11,
Germany, declare that:

I am the inventor of the invention embodied in the above-referenced application, and that I have
read and understand the application. The following is a description of experiments conducted by me or
at my direction in accordance with the present invention which provide evidence of the efficacy and
enablement of the present invention.

Animal Experiments

To our understanding, there is no chance for a high-efficiency immunostimulation by any

ATTORNEY DOCKET NO. 07038.0003U2
Serial No. Unsigned

biological response modifiers (BRMs) as long as the inevitably costimulated suppressor cells are not depleted. In an animal model (murine B16 melanoma model), we tested the combination of a tumor-specific immunostimulation with the depletion of potential suppressor cells.

To generate a tumor-specific immunostimulation, we injected donor mice with a mixture of tumor cells plus *B. subtilis* lyophilisate. This pretreatment of donor mice resulted in the generation of tumor-specific cytotoxic T cells (CTLs) within 14 days. These tumocidal CTLs were, however, accompanied by the costimulated suppressor cells.

To eliminate suppressor cells or their (co)inducers, we treated both the tumor-bearing mice recipients, ("R") and the donor mice (donors, "D") either by pan-T-specific or subset-specific monoclonal antibodies (Mabs) and their combinations.

In the experimental series S (S1...S6) the donor splenocytes were treated *ex vivo* by subset-specific Mabs and the recipient mice by the pan-T-specific Mab *in vivo*. In the experimental series N (N1...N6), both the donor splenocytes and recipients were treated by the same subset-specific Mabs.

The CD4 positive T cells ("helper/inducer T cells") can be considered as promoters of the CD4 positive effector suppressor cells. The so called "inducer" and "inducer/transducer" suppressor cells

ATTORNEY DOCKET NO. 07038.0003U2
Serial No. Unassigned

The idea behind the depletion of macrophages (alone or in combination with the T4 or T8 subset) was the (co)depletion of "suppressor monocytes" which are able to induce the suppressor T cells (via Th2 cells).

The tumor specific immune stimulation in recipient mice was generated by injecting splenocytes from donor mice which received a mixture of isolated melanoma tumor cells and *B. subtilis* lyophilisate. The use of donor splenocytes is analogous to the use of preactivated lymphocytes because syngeneic mice were used and because splenocytes of mice are similar to or at least comparable to lymphocytes. Splenocytes of syngeneic mice were used because it is too difficult to withdraw blood from mice and reinject it because mice do not have a high volume of blood. *B. subtilis* is used as an activator or immunostimulator.

Note: Because the suppressor-selective Mabs are not available, the suppressor-subsets could only be co-depleted along with other non-suppressor subpopulations.

Table 1: Survival data, expressed in the number of mice

	Ref1	Ref2	Ref3	S1	S2	S3	S4	S5	N1	N2	N3	N4	N5
d8	13/13	22/24	3/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
d10	11/13	17/24	2/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6
d32	0/13	2/24	1/6	4/6	3/6	4/6	5/6	2/6	4/6	4/6	2/6	6/6	2/6
d48	0/13	0/24	0/6	2/6	1/6	2/6	2/6	2/6	2/6	1/6	1/6	4/6	0/6
d64	0/13	0/24	0/6	2/6	1/6	2/6	1/6	2/6	2/6	1/6	1/6	2/6	0/6

ATTORNEY DOCKET NO. 07038.0003U2
Serial No. Unsigned

d8...d64 (day8...day64): indicates how many days after injection of donor effector cells *as tumor-specific immunosuppressors* (Ref3; S1-S5; N1-N5) or how many days after tumor excision (Ref1) and T_h elimination (Ref2), respectively. For example, d8 means 8 days after....

Ref1...only tumor excision

Ref2...tumor excision plus suppressor cell elimination (in recipient)

Ref3...tumor excision plus tumor-specific immunostimulation

S1-S5 and N1-N5...tumor excision plus suppressor cell elimination plus tumor-specific immunostimulation

Table 2: Survival data (in %)

	Ref1	Ref2	Ref3	S1	S2	S3	S4	S5	N1	N2	N3	N4	N5
d8	100	91,7	50	100	100	100	100	100	100	100	100	100	100
d16	84,8	70,8	33,3	100	100	100	100	100	100	100	100	100	100
d32	0	8,3	16,7	66,7	50	66,7	83,3	33,3	66,7	66,7	33,3	100	33,3
d48	0	0	0	33,3	16,7	33,3	33,3	33,3	33,3	16,7	16,7	66,7	0
d64	0	0	0	33,3	16,7	33,3	16,7	33,3	33,3	16,7	16,7	33,3	0

Note: Table 2 expresses survival data of the animals in terms of % of the group surviving.

Technical details

Part 1: Use of anti-pan T = anti-Thy1.2-Mab in recipient mice and subpopulation-specific Mabs for donor mice splenocytes

ATTORNEY DOCKET NO. 07038.0003U3
Serial No. Unassigned

lyophilisate (Sigma B4006). The mixture (B16-cells plus *B. subtilis*) has to be suspended in 0.05 ml of sterile PBS (or Hanks or RPMI 1640). If *B. subtilis* lyophilisate is not available in sufficient amounts, alternative bacterial lyophilisates (acetone powders) can be taken instead of the *B. subtilis* lyophilisate.

On day +14 after tumor inoculation, the spleens of these donors are removed. The removed splenocytes are treated *in vitro* by monoclonal antibodies (Mabs) to remove selectively the corresponding immunocyte subpopulation. This process thus removes suppressor cells induced in the donor so that when the donor splenocytes are injected into the recipient, no contaminating suppressor cells are introduced into the recipient after the recipient has already been treated to eliminate its own tumor suppressor cells.

In order to simplify the procedure, these subpopulations are only opsonized (*not depleted*) *in vitro*; their elimination occurs *in vivo* after the injection of (preopsonized) donor splenocytes into the recipients. This simplified procedure consists of the following steps: The anti-CD4 (CLO12A), the anti-CD8 (CL169) and the anti-B cells (MCA450)-Mabs have to be pre-diluted 1:10, the anti-monocyte-Mab (MCA519) 1:2 with sterile PBS (or Hanks or RPMI 1640), aliquoted for single mouse injection (0.200 ml) or for 6 mice-groups (1.200 ml) or for larger groups of daily injected mice (n x 0.200 ml) and thereafter frozen ($\sim 20^{\circ}\text{C}$ or $\sim 70^{\circ}\text{C}$) or kept at 0-4 $^{\circ}\text{C}$ under sterile conditions. In addition, the donor splenocytes are centrifuged (300 g, 5 min), resuspended in 0.200 ml of the above-mentioned Mab-solution, i.e., the 1:10 or 1:2 Mab-predilutions, incubated at 37 $^{\circ}\text{C}$ for 30 min and thereafter injected intravenously into the recipient mice.

of each Mab should be used.

Recipient mice: Mice are inoculated with 2×10^6 B16 tumor cells. On day +9 after tumor inoculation, the animals are injected with cyclophosphamide ($120 \text{ mg/kg} \approx 3 \text{ mg/25 g}$), and 1-3 hours before the tumor excision (on day +10 post-inoculation), all recipients are injected with anti-Thy 1.2 in exactly the same way and dose as practiced in other experiments.

- S1 Use of anti CD4 (CLO12A)-Mab for the treatment of donor splenocytes
- S2 Use of anti CD8 (CL169)-Mab for the treatment of donor splenocytes
- S3 Use of anti-macrophage (MCA519)-Mab for the donor splenocytes
- S4 Use of anti CD4 plus anti-macrophage-Mabs for the donor splenocytes
- S5 Use of anti CD8 plus anti-macrophage-Mabs for the donor splenocytes

Note: All recipients were treated *in vivo* by the pan-T-specific anti-Thy1.2-Mab.

Part II: Use of the same subpopulation-specific Mabs, both for donor splenocytes and in recipient mice

Comment: The only difference between the experiments of the "Part I" and those of the "Part II" is the replacement of the pan-T-specific, i.e., anti-Thy1.2-Mabs, in the "Part I" experiments by the subpopulation-specific Mabs in the "Part II" experiments. Therefore, the recipients of the type (R1) and (R2) are different in "Part I" and "Part II", whereas the donors (D1) and (D2) are identical in both experimental series.

ATTORNEY DOCKET NO. 07038.000302
Serial No. Unassigned

Donor mice: Identical with (D1) and (D2) donors from "Part I." The mice of D1 and the mice of D2 were the same kind of mice, but the animals used in D1 were different from the animals used in D2.

Recipient mice: Both recipient types (R1) and (R2) differ only in the replacement of anti-Thy.1.2-Mab (Part I) by subpopulation-specific Mabs (Part II), such as anti CD4-, anti CD8-, anti-macrophage, anti CD4- plus anti-macrophage, and anti CD8- plus anti-macrophage-Mabs.

- N1 Use of anti CD4 (CLO12A)-Mab for donor mice (*in vitro*) and the same anti CD4 (CLO12A)-Mab for recipient mice (*in vivo*)
- N2 Use of anti CD8 (CL169)-Mab for donor mice (*in vitro*) and of the same anti CD8 (CL169)-Mab for recipient mice (*in vivo*)
- N3 Use of anti-macrophage (MCA519)-Mab for donor mice (*in vitro*) and the same Mab for recipient mice (*in vivo*)
- N4 Use of anti CD4 plus anti-macrophage-Mabs for donor mice (*in vitro*) and of the same Mabs for recipient mice (*in vivo*)
- N5 Use of anti CD8 plus anti-macrophage-Mabs for donor mice (*in vitro*) and for recipient mice (*in vivo*)

In the present experiments, using the methods claimed, a significant improvement in the survival of the treated mice was observed. Thus, there is a very strong basis to believe that the methods as described herein and claimed will be effective to treat cancer.

ATTORNEY DOCKET NO. 07038.0003U2
Serial No. Unassigned

I declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any patent issuing therefrom.

February 27, 2002
Date

Dr Peter Leskovar
Peter Leskovar